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Antibacterial and antioxidative activity of the essential oil and seed extracts of *Artocarpus heterophyllus* for effective shelf-life enhancement of stored meat

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Abstract

This study was designed to examine the in vitro antimicrobial and antioxidant activity of the essential oil and various extracts processed from Jackfruit seed (*Artocarpus heterophyllus*). Seed extracts obtained from Jackfruit were tested for TPC and TFC, and the TPC of 34.48 ± 1.77 mg GAE/g extract was detected while TFC of 0.19 ± 0.004 mg QE/g extract was found significantly. Phytochemical screening of *A. heterophyllus* seed extracts using UPLC–QTOF/MS revealed the presence of eight compounds, representing (−)-Epiafzelechin-3-O-(6″-O-acetyl)-β-D-allosepyranoside was the most abundant phenolic compound. Further samples were also subjected to a screening for their possible antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl assay. DPPH analysis revealed that seed extract of *A. heterophyllus* has satisfactory radical scavenging capacity which could scavenge the free radicals produced during oxidation process in meat. The antioxidant activity can be observed for meat treated with seed extract as the TBA value was decreased in the samples on the addition of seed extract. However, in this study, seed extract treatment exhibited significant increase in MetMb content at 2 and 3 days of storage than in the control. The significance for all comparisons was determined at the $p < .05$ level. This result indicated that Jackfruit extract can be used commercially for meat shelf-life management.

Practical applications

Jackfruit seed extract is a natural preservative which improves the shelf-life storage of meat without causing any adverse effects. Natural preservative from plant source always better option when compare with chemical preservative. Jackfruit seed extracts could be used for shelf-life enhancement of stored meat in natural condition without affecting the quality as well as the taste of meat.

KEYWORDS

Artocarpus heterophyllus, DPPH assay, MetMb, UPLC–QTOF/MS

1 | INTRODUCTION

Meat, the muscle tissue of slaughter animals, is an indispensable source of nutrients in human diet and are consumed in massive amounts in developed and modern societies. Meat composed of water, proteins, lipids, essential amino acids and a small proportion of minerals, vitamins, and carbohydrates (Hopkins et al., 2014; Shah et al., 2014). The composition of meat can vary among different sources of animal and also the diet of the animal (Shahidi, 2016). Meat and meat products are susceptible to quality deterioration due to the prooxidant substances in meat like polyunsaturated fatty acids (PUFA), cholesterol, proteins, and pigments which are susceptible to oxidation (Devatkal et al., 2014; Holman et al., 2016). However, the major form of chemical deterioration is contributed by lipid oxidation that often occur during processing and storage (Lorenzo et al., 2014). Lipid oxidation is a rather multifaceted process and relies on chemical constituent of meat, light, oxygen access, and storage temperature (Kanner, 1994). During this process, primary and secondary oxidation products such as short-chain aldehydes, ketones, and other oxidized compounds will be developed (Kumar et al., 2015). The produced oxidation products have adverse effects on the overall quality and acceptability of meat and meat products causing alterations in nutritional quality as well as changes in sensory including color, texture, and flavor (Vaithiyanathan et al., 2011). Oxidative degradation of lipids can be delayed or suppressed by the use of antioxidants in meat and meat products and thus improve quality, nutritional value, and shelf life (Bhuyar, Rahim, Sundararaju, Maniam, et al., 2020).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) have been extensively used in meat and poultry products to uphold the safety and prolong the shelf life of meat and meat products (Huang et al., 2011). These synthetic antioxidants are used to control the lipid oxidation and to eliminate bacteria or molds. Though they both are powerful and inexpensive, synthetic antioxidants are suspected causing toxicological effects and consumer concern has led to a lessening in their usage in the food industry (Fernandes et al., 2018). Thus, directing at the decreasing of the consumption of synthetic chemical compounds that may impair the consumer's health and well-being has created demand for natural antioxidants by consumers and the meat industry. In this manner, natural antioxidants from plants extract can be used as alternatives to synthetic antioxidants owing to their comparable or superior effect on the inhibition of lipid oxidation and bacterial growth (Bhuyar, Rahim, Sundararaju, Ramaraj, et al., 2020; Bhuyar et al., 2019; Fernandes et al., 2016). The use of natural antioxidants in the food industry was preferable as natural products do not necessitate comprehensive safety testing prior to use. These natural antioxidants from plants include lotus (Huang et al., 2011), oregano, sage (Fasseas et al., 2008), black pepper, rosemary (Martínez et al., 2007; Sebranek et al., 2005) green tea, and grape seed (Bañón et al., 2007). However, natural antioxidants are often more expensive than synthetic antioxidants. As a consequence, many attention has been focused on the extraction of antioxidants from cheap materials or residual sources from agricultural processing industries for example, potato peel (Singh & Rajini, 2008), citrus by-product including pulp, peel, seeds (Bampidis & Robinson, 2006), and carrot residues (Eim et al., 2008).

Jackfruit which is scientifically known as *Artocarpus heterophyllus* is categorized as a shrub belonging to Moraceae family. This plant is native to India and is widely grown in tropical countries including Malaysia, Thailand, Indonesia, Sri Lanka, Brazil, and Philippines (Zhang et al., 2017). Jackfruit is considered as an enormous fruit with an average weight from 10 to 30 kg. Several aspects including cultivation, climate, and regional growth geography are important in determining jackfruit size and weight (Cheok et al., 2018). The health benefits of jackfruit including its functional, physiological, and medicinal characteristics has been reported previously (Swami et al., 2012). The ripe fruit of jackfruit has a unique flavor of yellow sweet bulbs and seeds. The edible bulbs of ripe fruits are consumed fresh or processed into canned products. However, the edible portion of the bulb usually make up only 30%–35% of the total fruit weight, whereas the rest of the skin (55%–62%) and seed (8%–10%) are considered as wastes (Cheok et al., 2018). Seeds are light brown in color, oval, or oblong ellipsoid or round in shape with white and crisp within. There may be up to 500 seeds which can be found in a single fruit with the size of 2–3 cm in length and 1–1.5 cm in diameter each seed (Jha & Srivastava, 2012). Jackfruit seed give remarkable antioxidant activity due to the presence of polyphenolic compounds including phenolics and flavonoids (Gupta et al., 2011). However, to the best of our knowledge, lack of study has addressed the use of jackfruit seed as readily available and inexpensive antioxidants in meat and meat products. Thus, this study was undertaken to investigate the antioxidant activity of jackfruit seed extract on raw meat. In addition, the main polyphenolic compounds of jackfruit seed were investigated. Also, the study endeavored to explore the antimicrobial effect of jackfruit seed extract on raw meat.

2 | MATERIALS AND METHODS

2.1 | Preparation of *A. heterophyllus* seed extract

The fresh seeds of *A. heterophyllus* were washed and cleaned to remove its pulp and slime. Then, the seeds were minced using food processor followed by extracted with distilled water at room temperature overnight. The extracts were then freeze-dried, pulverized into fine powder, and stored at 4°C.

2.2 | Determination of total phenolics and flavonoids in the extracts

Total phenolics content (TPC) of seed extract (0.1 g) was performed using the Folin–Ciocalteu assay. Folin–Ciocalteu reagent was used as the reactive agent and gallic acid as a standard (Ramli et al., 2020). The absorbance of the reaction formed was measured at 760 nm against a blank. Result of TPC was expressed as grams of gallic acid

equivalents per gram of extract. Total flavonoid content (TFC) of seed extract (0.1 g) was done by colorimetric assay. A calibration curve was generated with Rutin as the reference compound, and the results were expressed as grams of Rutin equivalents per 100 g of dry extract. The results were expressed in milligrams of Rutin equivalents per gram of extract (Huang et al., 2011; Saengsawang et al., 2020).

2.3 | Phenolic profiles by UPLC–QTOF/MS

The phenolic compounds profiling of *A. heterophyllus* seed was accomplished using a Waters ACQUITY UPLC I-Class/Xevo in line with a Waters Xevo G2 Q-TOF mass spectrometer (Milford, MA, USA). Sample separation was conducted on a Zorbax Eclipse Plus Acquity UPLC BEH C18 (1.7 μm particle size) 2.1 mm × 50 mm. The temperature of column was maintained at 40°C. The flow rate was 0.60 ml/min, and the injection volume was 2 μl. A linear gradient elution of water, containing 0.1% formic acid (A) and acetonitrile (B) were used. The gradient was initiated with increasing to 5% B within 1 min, 5% B and then, linearly increased to 15% B within 2 min, then 15% B increased to 20% A within 3 min, 20% B increased to 30% B within 4 min, and 30% B increased to 35% B within 0.5 min. Next, 35% B increased to 43% B within 3 min and 43% B was increased to 50% B within 1.5 min. Finally, 50% B increased to 95% B within 3 min and 95% B increased to 100% B within 1 min. The MS spectra were acquired in positive and negative ion mode with an electrospray ionization (ESI) source. The data were scanned for each test sample from 50 Da to 1,500 Da. Nebulizing gas and collision gas are utilized using highly purified nitrogen (N_2) and ultra-high pure helium (He), respectively. In terms of positive electrospray mode, the capillary voltage was set at 1,500 V. The other source parameters were set as follows: source offset, 100 V; desolvation temperature, 550°C; cone gas flow, 50 L/h; cone temperature 120°C, and desolvation gas flow, 800 L/h.

2.4 | Preparation of meat samples

Fresh bovine meat was obtained from local market and only meat without fat was selected. The five meat samples were cut into subsamples of similar size (1 g weight with 1-inch diameter) before immersed with 3% (w/w) of seed extract. Only one sub sample was selected for each reaction assay. The experiment was carried out three times on the same day to avoid human error. No extract was added to the control sample. All meat samples were stored at 4°C in the dark for 24 hr and then, subjected to antioxidant activity assay for days 1, 2, and 3.

2.5 | Assessment of lipid oxidation

The TBA (thiobarbituric acid) assay is one of the most commonly used method for the assessment of lipid oxidation. The TBA test followed the method described with minor alteration (Fasseas et al., 2008). Briefly, 0.03 g of samples were mixed with a solution containing 0.6 ml of distilled water, 0.9 ml of phosphoric acid at (pH 2.0), and 0.9 ml of 0.8% (w/w) of TBA (diluted in 1.1% (w/w) sodium dodecyl sulfate (SDS)) in a test tube. Then, the mixture was vortexed and heated at 100°C for 60 min in a water bath. The mixture was cooling down prior to the addition of 3 ml butan-1-ol. The mixture was mixed well followed by centrifugation at 8,900 ×*g* for 10 min. The absorbance of the upper layer was determined at 532 nm using butan-1-ol solvent as a blank. As for result, the value was expressed as TBA values.

2.6 | DPPH analysis

To estimate the antioxidant activity of seed extract on meat sample, radical scavenging activity method was employed. The stable free-radical 2,2′-diphenylpicrylhydrazyl (DPPH) in radical scavenging or hydrogen donating ability was evaluated by the DPPH assay using the method described (Tepe et al., 2005). Briefly, 0.03 g of sample was mixed with 3 ml of 0.004% methanol solution of DPPH in a test tube at room temperature for 30 min. Then, the sample was centrifuged at 1,400 ×*g* for 10 min. Absorbance of the supernatants was read against a blank (methanol) at 517 nm. Measurements were expressed as absorbance and reducing values specified increasing antioxidant activity.

2.7 | Reducing power assay

The reducing power of sample was determined by the slight modification of the method described (Barros et al., 2007). Substances with reduced potential will react with potassium ferricyanide (Fe^{3+}) that lead to potassium ferrocyanide (Fe^{2+}) formation. Fe^{2+} produced will then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Marinated meat sample (0.03 g) was mixed with 2.5 ml of phosphate buffer (200 μM, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v) followed by incubation at 50°C for 20 min.After the incubation period, 2.5 ml of TCA (10%, w/v) was added and this mixture was centrifuged at 3,000 × g for 10 min. The resulting supernatant was mixed with 2.5 ml of deionized H_2O and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm (Barros et al., 2007). Increase in absorbance of the reaction indicated the increasing reducing power.

2.8 | Measurement of metmyoglobin

The concentration of Metmyoglobin (metMb) in meat sample was determined according to the method of Naveena et al. (2006) with some modifications. Briefly, a total amount of 0.5 g of meat sample was homogenized and mixed with 3 ml of cooled 0.04 M

phosphate buffer (<4°C) at pH 6.8. The mixture was stored for an hour at 4°C followed by centrifugation at 2,200 ×*g* for 5 min at 4°C. The supernatant was subjected to absorbance measurement at 503 nm.

2.9 | Determination of pH

For the measurement of pH, 0.5 g of samples were homogenized with 3 ml of distilled water. The homogenate was subjected to centrifugation at 2,240 ×*g* for 5 min. The resulting supernatant was filtered, and the pH was measured with pH meter at room temperature the pH meter was calibrated with standard buffer tablets (pH-4, pH-7.2, and pH-9) prior to use (Biomed, UK).

2.10 | Antibacterial screening

2.10.1 | Preparation of meat model medium

Meat model medium was prepared following the recommendations of (Ballester-Costan et al., 2017). A total amount of 10 grams of minced beef was added to 90 ml of one-quarter-strength buffered peptone water (pH 7.2) in a homogenizer and homogenized until smooth. Subsequently, the samples were filtered through a paper disk Whatman No 2 to discard solid particles and attained a clarified extract. Meat model medium was made mixing the extracts obtained from minced beef with agar solution in order to obtain a final solid medium solution with 1.5% agar. Finally, all prepared meat solutions were autoclaved at 121°C for 15 min prior to use.

2.10.2 | Disk diffusion method

Screening of seed extract for antibacterial activity was determined by the agar diffusion method according to the method described by Tepe et al. (2005). A standardized inoculum suspension of selective bacteria (0.1 ml of 106 CFU/ml) was spread on the solid meat model medium plates. The inoculums were allowed to dry for 5 min. Then, a sterile filter paper disk (9 mm in diameter) was impregnated with 30 μl of seed extract. The plates were incubated at room temperature for 15 min to permit the diffusion of the seed extract happen followed by incubation at 37°C for 24 hr. Afterward, the diameter of the clear zone around the disk was measured with a caliper and expressed in millimeters (disk diameter included) as its antimicrobial activity. All tests were performed in triplicate.

2.11 | Statistical analysis

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), Lipid oxidation, DPPH, reducing power assay, MetMb, and pH all the experiments comparatively studied interaction between the fixed terms like (i) control sample without *A. heterophyllus* seed extract marination and (ii) treated sample with *A. heterophyllus* seed extract marination for 24 hr were carried out on the same day. All the tests were performed for three times to avoid human error. The five different samples were used as random term for analysis. All the resulted data (mean values) were statistically analyzed with the SPSS analysis of variance (SPSS version 12.0 for Windows, SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used for the study of studentized range distribution in order to determine the critical values for comparisons between means (Biffin et al., 2019). The significance for all comparisons was determined at the $p < .05$ level.

3 | RESULTS AND DISCUSSIONS

3.1 | Total phenolics and total flavonoids in the seed extract

Seed extracts obtained from *A. heterophyllus* were tested for TPC and TFC, and the ratios of TFC/TPC were also calculated. The TPC of 34.48 ± 1.77 mg GAE/g extract was detected while TFC of 0.19 ± 0.004 mg QE/g extract was found significantly. The ratio of TFC/TPC was found to be 2.39.

3.2 | UPLC–QTOF/MS phenolic profiles

Ultra-performance liquid chromatography combined with time-of-flight mass spectrometry (UPLC–QTOF/MS) become a reliable analytical approach that can be used for phytochemical detection of plant extracts (Khammee et al., 2020; Nor et al., 2020). This approach involves the application of a small particle sized column of UPLC together with hypersensitive and high mass resolution TOF analyzer which offers operative complex samples separation together with broad numbers of ions and molecular formulas (Baek et al., 2012). Phytochemical screening of *A. heterophyllus* seed extracts using UPLC–QTOF/MS revealed the presence of 3,4-O-Dicaffeoylquinic acid, (–)-Epiafzelechin-3-O-(6″-O-acetyl)-β-D-allosepyranoside, Darendoside A, Sibiricaxanthone B, Kushenol R, 5,7,2′-Trihydroxy-flavanone-4′-O-β-D-glucoside, Dihydrokaempferol-5-O-β-D-glucopyranoside, and 2′-Hydroxy-3′,4′-dimethoxy-isoflavan-7-O-β-D-glucoside. The detection of phenolic compounds was determined elicited from the pattern of mass fragmentation, ion response, and lower mass error (acceptance range ± 5). In addition, satisfactory number of fragments detected by Mass Fragment were more than three. Based on the results processed in UNIFI software, the data of ± 5 mDa error is categorized as a good match while the data with ± 10 mDa error is considered as a poor match. The summary of the phenolic compounds identified in the seed extract of *A. heterophyllus* is presented in Table 1. According to the table, (–)-Epiafzelechin-3-O-(6″-O-acetyl)-β-D-allosepyranoside was the most abundant phenolic compound detected.

3.3 | Assessment of lipid oxidation

TBA assay is normally utilized to assess lipid oxidation value in meat and meat products (Gray & Monahan, 1992). Lipid oxidation is always associated with food quality deterioration including rancidity and off-flavor formation as well as the formation of potentially toxic and carcinogenic products which could impair the nutritive value and safety of foods. Oxidation of lipids can occur during the storage of meats and other fat-containing foods such as milk, oils, and nuts (Kanner, 1994). The development of MDA (malondialdehyde), aldehydic products produced from lipid oxidation, can be assessed during the TBA reaction. TBA analysis of untreated meat samples (control) represent higher value ($p < .05$) compared to meat sample treated with seed extract as shown in Figure 2(a). This implies that oxidation levels in the seed treatment were lower than in the control after day 3. The oxidation level increased with storage time for both treated and untreated meat samples (from day 1 to day 3). However, the antioxidant activity can be observed for meat treated with seed extract as the TBA value was decreased in the samples on the addition of seed extract.

3.4 | DPPH analysis

The assessment of antioxidant capacity can be measured through their free radical scavenging activity. Antioxidant elements can inhibit the detrimental action of the free radicals produced during the lipid peroxidation process by scavenging the free radicals and allow the detoxifying process (Zhang et al., 2017). DPPH method is a simple and rapid antioxidant assay used to investigate the free radical scavenging potential of antioxidant compounds. During the reaction, the alcoholic DPPH solution is reduced in the existence of hydrogen donating antioxidants thus lead into the development of colorless ethanol solution (Garcia et al., 2012). According to the data generated from the DPPH assay, the meat treated with seed extract of *A. heterophyllus* produced considerably ($p < .05$) effective antioxidant activity by showing lower absorbance compared to the control sample as presented in Figure 2(b). The antioxidant activity of the treated samples was significantly better than the untreated sample (control) from day 1 to day 3 of storage as observed in Figure 1. DPPH assays indicated that seed extract treatment of the meat samples significantly increased radical scavenging. Similar findings were also reported for the radical scavenging effect of natural substances from lotus (Nelumbo nucifera) rhizome knot and leaf (Huang et al., 2011) and Clove Oil and Clove Oleoresin (Sultana et al., 2017) in meat samples during storage.

3.5 | Reducing power assay

Reducing power activity work as a significant reflection of the antioxidant capacity. Results from the reducing power assay (Figure 2(c)) showed that the reducing power of the seed extract treatment was increased significantly ($p < .05$) compared to the control sample from day 1 to day 2 of storage. Increased absorbance of the reaction mixture specifies greater reducing power. This reductive ability might be due to the presence of the polyphenolic content in the seed extract of *A. heterophyllus*. However, the reduction potential of the seed extract treatment seemed to be decreased after day 3 of storage. The reducing power in the meat samples were measured by the conversion of Ferric cyanide complex (Fe^{3+}) to ferrous form (Fe^{2+}) in the presence of the reductants (antioxidants) and can be examined by determining the development of Perl's Prussian blue at 700 nm (Chung et al., 2002). Previously, several studies described the effectiveness of the natural plant extracts as a natural antioxidant which contain significant reducing power activity (Carmen et al., 2017; Huang et al., 2011; Kanatt et al., 2007).

3.6 | Measurement of metMb

The formation of MetMb depending on numerous factors such as oxygen partial pressure, pH, reducing power activity, temperature, and in some cases microbial growth (Mancini & Hunt, 2005).

TABLE 1 summary of phenolic compounds identified in the seed extract of *A. heterophyllus* using UPLC–QTOF/MS

Component name	Mass error (mDa)	Observed RT (min)	Response	Observed neutral mass (Da)	Total fragments found
3,4-O-Dicaffeoylquinic acid	1.8	2.33	1,017	516.1286	8
(−)-Epiafzelechin-3-O-(6″-O-acetyl)-β-D-allosepyranoside	−1.2	3.65	3,494	478.1463	35
Darendoside A	0.5	4.32	3,415	432.1636	4
Sibiricaxanthone B	−0.3	4.49	7,502	538.132	5
Kushenol R	−1.3	4.56	14,245	422.208	5
5,7,2′-Trihydroxy-flavanone-4′-O-β-D-glucoside	−1.3	4.72	18,701	450.1149	6
Dihydrokaempferol-5-O-β-D-glucopyranoside	0	5.26	2,506	450.1162	6
2′-Hydroxy-3′,4′-dimethoxy-isoflavan-7-O-β-D-glucoside	−1.9	7.39	16,194	464.1664	3

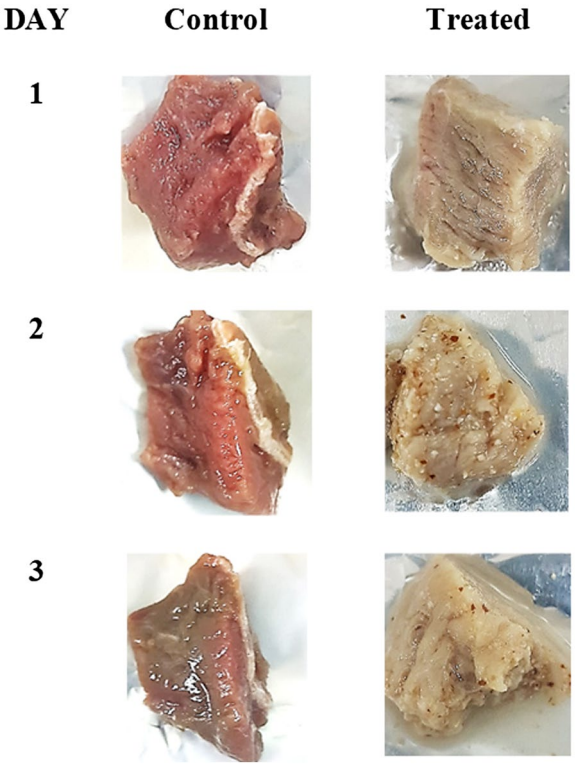

FIGURE 1 Antioxidant activity levels in the control and marinated meat samples

Metmyoglobin is a result of oxidation of pigment which lead to discoloration of the meat. Oxygenation of DeoxMb to O_2Mb produce the redness color of meat due to exposure to oxygen, O_2. But, according to the partial pressure of oxygen, O_2 (pO_2), the oxygenation is considered reversible. The result from the metMb assay of the meat samples, the seed extract treatment significantly ($p < .05$) retarded the accumulation of metMb (i.e., lower absorbance) in day 1 compared to the control and significantly increased after that (Figure 2(d)). It was reported that the free radical scavengers could inhibit the formation of metMb. Mb in fresh meat can be divided into three types such as deoxymyoglobin [deoxy (Fe2+)Mb], oxymyoglobin [oxy(Fe2+)Mb], and metmyoglobin [met (Fe3+)Mb] (Suman & Joseph, 2013). DPPH analysis revealed that seed extract of *A. heterophyllus* has satisfactory radical scavenging capacity which could scavenge the free radicals produced during the oxidation process in meat. However, in this study, seed extract treatment exhibited showed significantly increased MetMb content at 2 and 3 days of storage than in the control. The higher absorbance at 503 nm might be a consequence of the natural color (light yellow) of the seed extract treatment solutions. This results are in accordance with the observations of (Bekhit et al., 2003) and (Zahid et al., 2018), who showed that the meat samples treated with the natural antioxidant increased the accumulation of MetMb during storage period.

3.7 | Determination of pH

There was reduction in pH for treated meat compared to control which slightly increase as observed in Figure 3. The pH of marinate sample at Day 1 was significantly higher than the control with pH 5.23 for marinate and pH 4.7 for control. Later, on the Day 2, the pH of control was slightly higher than marinate with pH 4.69 for control

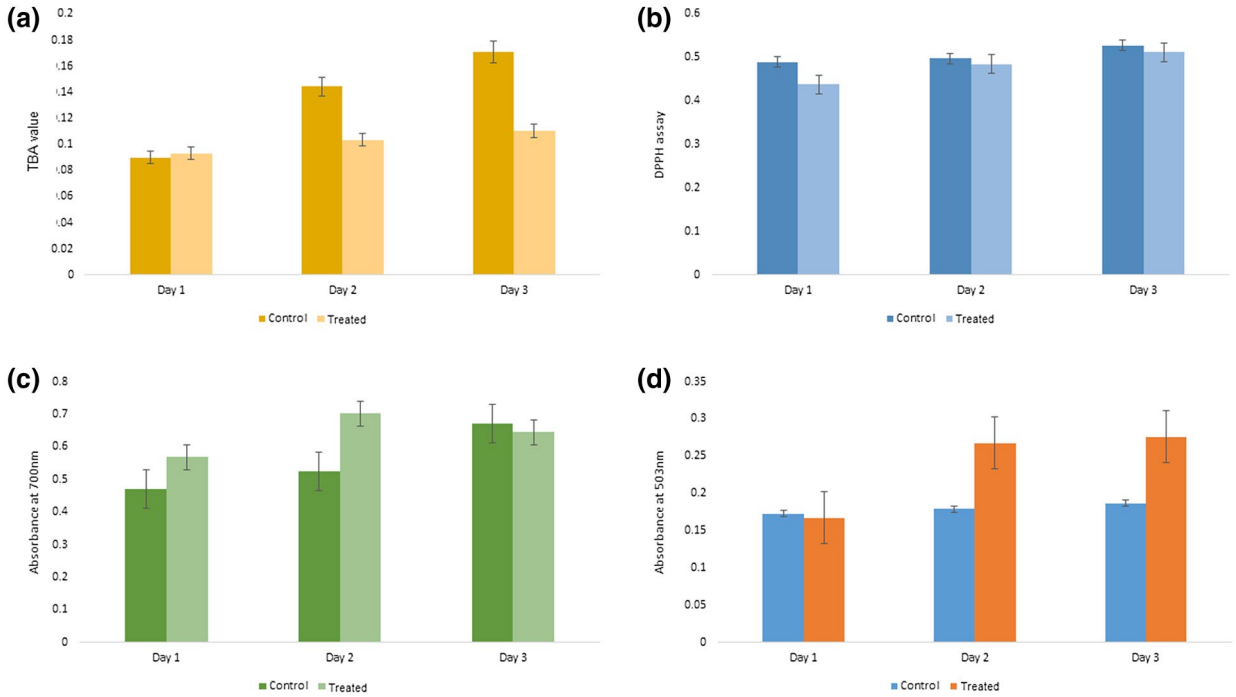

FIGURE 2 Antioxidant activity levels in the meat samples on storage, as determined by the (a) TBA assay, (b) DPPH assay, (c) reducing power assay (d) metmyoglobin assay. (Data represent means ± respective standard errors)

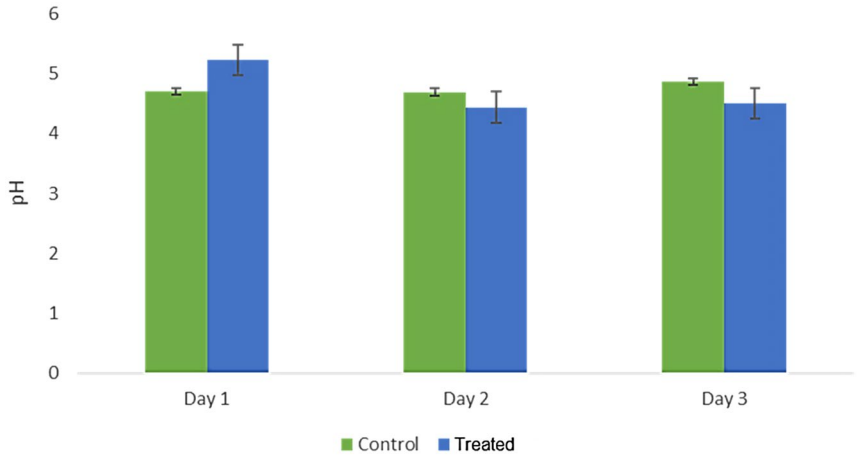

FIGURE 3 pH of samples on storage from day 1 to 3. (Data represent means ± respective standard errors)

TABLE 2 Antibacterial activity of *Passiflora edulis* peels against several bacterial strain using meat extract as culture medium

	Diameter of Inhibition Zone (mm) Including Disk (6 mm)			
	E. coli	*S. marcescens*	*P. aeruginosa*	*S. aureus*
Negative control (dH_2O)	(–)	(–)	(–)	(–)
Positive control (Ampicillin)	$30 \pm 2.51^{(+++)}$	$26 \pm 1.15^{(++)}$	$25 \pm 1.68^{(++)}$	$27 \pm 1.52^{(++)}$
Seed extract disk	$13 \pm 1.52^{(-)}$	$17 \pm 0.57^{(+)}$	$19 \pm 0.83^{(+)}$	$21 \pm 1.73^{(++)}$

and 4.44 for marinate. However, on Day 3, the pH for marinate is slightly increased to pH 4.5 and for control which is at pH 4.87. Based on all 3 days, the pH of the control meat seems to be consistent with just slight decrease and increase about ± 0.1. The marinated sample tends to make the meat sample to become more acidic. Hydrolysis of the muscles by seed extracts causing the reduction of the pH as the amino acids were released into the system. This is due to the water movement from the myofilament space into extracellular space and, therefore, led myofibrillar to shrink. Changes of pH value were also due to the postmortem metabolism and also due to the substances that were added to the meat during technological process (Gault, 1985).

3.8 | Antibacterial activity

The agar disk diffusion method was used to determine the antibacterial activity of fresh seed of Jackfruit (*Artocarpus heterophyllus)* extract against gram-positive and gram-negative bacteria (Bhuyar, Sathyavathi, et al., 2020; Bhuyar, Yusoff, et al.,2020; Bhuyar et al., 2018). *A. heterophyllus* extract showed antimicrobial activity against four types of bacteria that commonly involved with refrigerated food storage. Table 2 shows the antibacterial of jackfruit seed extract using meat extract as culture medium. The results obtained from the disk diffusion method indicated that *A. heterophyllus* seed extract exerted a strong antibacterial activity against four tested strains which were *E. coli*, *S. marcescens*, *P. aeruginosa*, and *S. aureus*. The largest clearance of inhibition zone from peel extract is *S. aureus* species. In Table 2 below showed p value between all types of bacteria is ($p > .05$).

The antimicrobial activities observed in this study might be due to the presence, as occurred with the antioxidant activity, of phenolic acid and/or flavonoid compounds. Extracts of various fruits and fruits co-products containing phenolic and flavonoids have previously been reported to possess antimicrobial activity (López-vargas et al., 2013). However, although the extracts of various fruits and fruits co-products show antimicrobial activity, the reason behind this capacity is not well documented. Several authors have suggested that the antibacterial activity of phenolic acids and flavonoids may be attributable to the cytoplasmic membrane damage caused by perforation and/or a reduction in membrane fluidity (Vaithiyanathan et al., 2011), the inhibition of energy metabolism (Carmen et al., 2017), or the inhibition of nucleic acid synthesis (Jha & Srivastava, 2012).

4 | CONCLUSION

This research constitutes the first assessment of antibacterial and antioxidant potential along with the comprehensive identification of phytochemical compounds of jackfruit seed extract. Comparing with jackfruit pulp and flack, seed extract gave the highest TPC and TFC with the value TPC of 34.48 ± 1.77 mg GAE/g extract was detected while TFC of 0.19 ± 0.004 mg QE/g

extract was found significantly. The strongest radical scavenging ability and antibacterial inhibition were also detected in jackfruit seed extract. Eight compounds were tentatively characterized with HPLC-QTOF-MS/MS, including the presence of 3,4-O-Dicaffeoylquinic acid, (-)-Epiafzelechin-3-O-(6″-O-acetyl)-β-D-allosepyranoside, Darendoside A, Sibiricaxanthone B, Kushenol R, 5,7,2′-Trihydroxy-flavanone-4′-O-β-D-glucoside, Dihydrokaempferol-5-O-β-D-glucopyranoside, and 2′-Hydroxy-3′,4′-dimethoxy-isoflavan-7-O-β-D-glucoside. The summary of the phenolic compounds identified in the jackfruit seed extract (–)-Epiafzelechin-3-O-(6″-O-acetyl)-β-D-allosepyranoside is the most abundant phenolic compound detected. DPPH analysis revealed that seed extract of *A. heterophyllus* has satisfactory radical scavenging capacity which could scavenge the free radicals produced during oxidation process in meat. This research indicated that jackfruit seed could be a promising source of natural antioxidants, the results can also provide useful information for the further functional research of jackfruit seed and rapid identification of bio-active compounds from other plant materials. But further researches are needed to elucidate the delicate structure of which contribute to the tested bio-activities, along with the in vivo antioxidant and hypoglycemic activity.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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